MicroBioTest Protocol

Efficacy Evaluation of Field Use Copper Enhanced Hard Surfaces as a Sanitizer

Testing Facility
MicroBioTest
Division of Microbac Laboratories, Inc.
105 Carpenter Drive
Sterling, VA 20164

Prepared for
Cupron Inc.
Suite 123
800 East Leigh Street
Richmond, VA 23219

October 3, 2014

MicroBioTest Protocol: 619.1.10.03.14

MicroBioTest Project: 619 - 140

OBJECTIVE:

This test is designed to substantiate effectiveness claims for a substance containing copper with sanitizing claims intended to be registered with the Environmental Protection Agency as an inanimate hard surface other than those that come in contact with food or beverages. The test is consistent with the EPA Test Method for Efficacy of Copper Alloy Surfaces as a Sanitizer with the exception that only *Staphylococcus aureus* will be evaluated. This test specifically incorporates the evaluation of test carriers that have been in field use for at least one year.

TESTING CONDITIONS:

A total of five test replicates will be evaluated using carriers prepared from a copper enhanced hard surface. One lot of the test surface will be evaluated. Prepared carriers of the test surface will be inoculated with *Staphylococcus aureus*, held for the stipulated contact time, transferred to a neutralizing solution and mixed. Dilutions of the neutralizer will be plated, incubated, and observed for growth.

MATERIALS:

A. Test and control surfaces supplied by the sponsor: (see last page for details).

Test and control carriers: 1" x 1" coupons, also referred to as carriers

- The identity, strength, purity, and composition, or other characteristics which will appropriately define the test, control, or reference surfaces shall be determined for each batch and shall be documented by the sponsor before its use in a study. Methods of synthesis, fabrication, or derivation of the test, control, or reference surfaces shall be documented and retained by the sponsor.

- When relevant to the conduct of the study the solubility of each test, control, or reference agent shall be determined by the sponsor before the experimental start date. The stability of the test, control, or reference agent shall be determined by the sponsor before the experimental start date or concomitantly according to written standard operating procedures, which provide for periodic analysis of each batch.

The test and control surfaces will be tested as supplied by the sponsor unless directed otherwise. All operations performed on the surfaces such as dilution or specialized storage conditions must be specified by the sponsor before initiation of testing.

The sponsor assures MicroBioTest, Division of Microbac Laboratories, Inc. (MicroBioTest) testing facility management that the test surface has been appropriately tested for identity, strength, purity, stability, and uniformity as applicable.

MicroBioTest will retain all unused test and control surfaces after completion of the test, and then only discard them with client permission in a manner that meets the approval of the safety officer.

B. Materials supplied by MicroBioTest including but not limited to:

1. Challenge microorganism, required by the sponsor: *Staphylococcus aureus*, ATCC 6538

2. Media and reagents:
   a. Tryptic Soy Broth (TSB)
   b. Neutralizer: 2X Letheen Broth
   c. Phosphate Buffer Saline dilution blanks (PBS)
   d. Tryptic Soy Agar (TSA)
   e. Heat-inactivated Fetal Bovine Serum (FBS)
   f. Triton X-100 solution (1% solution)
   g. Sterile deionized water
   h. 70-85% Isopropyl alcohol

3. Miscellaneous laboratory equipment and supplies as required

TEST SYSTEM IDENTIFICATION:

All test and control tube racks will be labeled with microorganism, test agent (if applicable) and project number prior to initiation of the study and during incubation. Petri dishes will be labeled with microorganism prior to initiation of the study and microorganism and project number during incubation.

EXPERIMENTAL DESIGN:

A. Inocula preparation:

Bacteria from stock cultures will be transferred into TSB and incubated at 35-37°C for 24±2 hours. Daily transfers will be made for at least three consecutive days (but no more than 10 days). For each transfer, tubes containing 10 mL of TSB will be inoculated using two loopfuls (4-mm inside diameter) of inoculum for each tube. A 48±4 hour culture will be used for the inocula on the day of testing.

Transfers more than 15 days away from the stock cultures will not be used for the inocula for the test.

Each culture will be thoroughly mixed on a vortex-mixer and allowed to settle for ≥15 minutes. The upper two-thirds of each culture will be aspirated and used as the inoculum.

B. Addition of organic load:

A 0.25 mL aliquot of FBS plus 0.05 mL of a 1% Triton X-100 solution to 4.70 mL of bacteria suspension to yield a 5% FBS and 0.01% Triton X-100 soil load.

C. Test and Control Carrier preparation:

The test (one lot, five replicates) and control surfaces/carriers (three replicates) plus additional test and control surfaces as required for the remaining controls will be cleaned by submersion in 70-85% in Isopropyl alcohol, rinsed with sterile deionized water, and allowed to air dry.

After drying completely, the carriers will be steam sterilized for 15 minutes at 121°C. The carriers will be allowed to cool and held at ambient room temperature until use. Prior to use, each carrier will be aseptically transferred into plastic Petri dishes (one dish for each carrier) matted with two pieces of filter paper using sterile forceps.

D. Carrier inoculation:

A 0.02 mL aliquot of the inoculum will be transferred onto each sterile carrier using a calibrated micropippetor. The inoculum will be spread to within approximately 1/8" of the edge of the carrier. The carriers will be allowed to dry with lids ajar for 20-40 minutes under ambient conditions. The exposure period (contact time) begins immediately after drying.

E. Test:

Five inoculated and dried carriers will be held for the exposure (contact) time. The contact time will begin immediately after drying in accordance with Section D, Carrier inoculation.

At the conclusion of the contact time, each carrier will be transferred to a jar containing 20 mL of neutralizer at the appropriate staggered intervals. Each jar will be sonicated for five minutes and then rotated by hand to mix. Within one hour after sonication, serial dilutions will be prepared using PBS ($10^{-1} - 10^{-4}$). Duplicate 1.0 mL aliquots from each jar/dilution ($10^{0} - 10^{-4}$) will be plated using TSA pour plates.

Plates will be incubated for 48±4 hours at 35-37°C, colonies will be counted and CFU/carrier calculated.

F. Controls:

1. Carrier quantitation control:

A parallel control will be run using the control carriers (surfaces) in the same manner as the test (including the contact time) with the exception that three replicates will be evaluated rather than five. All plates will be incubated appropriately in the same manner as the test plates.

2. Culture purity control:

The prepared culture will be streaked for isolation using TSA. All plates will be incubated appropriately in the same manner as the test plates. The isolated cultures will be observed for purity.

3. Organic soil sterility control:

   Duplicate 1.0 mL aliquots of the prepared organic soil will be plated in TSA pour plates. The plates will be incubated for 48±4 hours at 35-37°C and observed for growth or no growth.

4. Inoculum confirmation counts control:

   The prepared inoculum will be serially diluted using PBS and selected dilutions will be plated in duplicate using TSA pour plates. All plates will be incubated appropriately in the same manner as the test plates.

5. Neutralizer sterility control:

   A single jar of containing the neutralizer will be incubated for 48±4 hours at 35-37°C. The neutralizer will be observed for growth or no growth.

6. Carrier sterility control:

   An uninoculated test and control carrier will be subcultured into independent jars containing the neutralizer and incubated for 48±4 hours at 35-37°C. The neutralizer will be observed for growth or no growth.

7. Carrier viability control:

   A single inoculated control carrier will be subcultured into a jar containing the neutralizer and incubated in the same manner as the test plates. The neutralizer jars will be observed for growth or no growth.

8. Neutralizer effectiveness control:

   A single sterile test carrier will be neutralized in the same manner as the test (transferred into individual jars containing 20 mL of neutralizer. To each jar, a 1.0 mL aliquot of the diluted inoculum will be added to yield ≤100 CFU/mL in the neutralizer. The jar will be mixed and a 1.0 mL aliquot will be removed and plated in duplicate.

   A numbers control will be performed in the same manner with the exception that a sterile control carrier will be used. All plates will be incubated in the same manner as the test plates.

9. Microorganism confirmation procedures:

A randomly selected colony from the carrier quantitation control plates, and if applicable, a randomly selected colony from a test plate will be confirmed by colony morphology and Gram stain according to extant SOPs. The same procedures will be performed using the culture purity control plates and the result regarding purity will be documented as well.

TEST ACCEPTANCE CRITERIA:

The test will be acceptable for evaluation of the test results if the neutralizer is effective and non-toxic. The study director may consider other causes that may affect test reliability and acceptance. There are no proposed statistical methods for this test.

- The average recovery for the Carrier Quantitation Control must be at least 2.0×10^4 CFU/carrier.
- The CFU recovered for the neutralizer effectiveness controls should be within 1.0 $\log_{10}$ of the parallel neutralization confirmation control.
- The carrier sterility controls must exhibit no growth.
- The carrier viability controls must exhibit growth.
- The purity controls must demonstrate pure cultures.
- The organic soil sterility control must exhibit no growth.
- The neutralizer sterility control must exhibit no growth.

PRODUCT EVALUATION CRITERIA:

According to EPA guidelines, the test agent meets effectiveness requirements, if the test results exhibit a bacterial reduction of at least 99.9% over the Carrier Quantitation Control. Please note that this study is only evaluating the effectiveness of the test agent for *Staphylococcus aureus*.

DATA PRESENTATION:

The final report will include the following information in tabular form:

- The average colony-forming units (CFU)/carrier and percent reduction for each evaluation.
- The results for all the controls.

PERSONNEL AND TESTING FACILITIES:

A study director will be assigned before initiation of the test. Resumes for technical personnel are maintained and are available on request. This study will be conducted at MicroBioTest, 105 Carpenter Drive, Sterling, VA 20164.

REPORT FORMAT:

MicroBioTest employs a standard report format for each test design. Each final report provides the following information:

- Sponsor identification and test agent identification
- Type of test and project number
- Dates of study initiation and completion
- Interpretation of results and conclusions
- Test results
- Methods and evaluation criteria
- Signed Quality Assurance and Compliance Statements (for GLP studies, if provided by the sponsor)

CONFIDENTIALITY:

All data generated at MicroBioTest are held in strictest confidence and are available only to the sponsor and the sponsor designated authorities (if applicable). In turn, no reference to MicroBioTest's promotion of the evaluated test articles may be made public by the sponsor.

REGULATORY COMPLIANCE AND QUALITY ASSURANCE (applicable to GLP studies only)

This study will be performed in compliance with the US Environmental Protection Agency's Good Laboratory Practices regulations, 40 CFR 160. Note: information on the identity, strength, purity, stability, uniformity, and dose solution analysis of the test agent resides with the sponsor of the study unless otherwise stated.

The Quality Assurance Unit of MicroBioTest will inspect the conduct of the study for GLP compliance. The dates of the inspections and the dates that findings are reported to the study management and study director will be included in the final report.

RECORDS TO BE MAINTAINED:

All raw data, protocol, protocol modifications, test agent records, final report, and correspondence between MicroBioTest and the sponsor will be stored in the archives at MicroBioTest, 105 Carpenter Drive, Sterling, Virginia 20164 or in a controlled facility off site.

All changes or revisions to this approved protocol will be documented, signed by the study director, dated and maintained with this protocol. The sponsor will be notified of any change, resolution, and impact on the study as soon as practical.

The proposed experimental start and termination dates; additional information about the test agent; challenge microorganism used; media and reagent identification; and the type of neutralizers employed in the test will be addressed in a project sheet issued separately for each study. The date the study director signs the protocol will be the initiation date. All project sheets will be forwarded to the study sponsor.

MISCELLANEOUS INFORMATION:

The following information is to be completed by sponsor before initiation of study:

A. Name and address: Cupron Inc.
Suite 123
800 East Leigh Street
Richmond, VA 23219

B. Test surface information:

Test surface name	18 month field use Sample
Lot No.	0001-001
Manufacture date	02/2013
Field Use Period	18 months
Active ingredient	Copper Oxide
Control surface	The sponsor will also provide control surfaces that will not contain any antimicrobial active ingredient (Cupron Control Hard Surfaces).

C. Test conditions:

Contact time: 2 hours

Exposure temperature: Ambient room temperature (20±1C)

D. Organic load – serum added to achieve 5% in the inoculum: ■ yes ☐ no

E. Precautions/storage – MSDS or certificate of analysis provided: ☐ yes ■ no

REPORT HANDLING AND STUDY CONDUCT: CAL DPR, GLP

PROTOCOL APPROVAL:

Sponsor Signature: _____ Date: 10/3/2014
Alastair B. Monk, PhD

Study Director Signature: _____ Date: 10/6/14
Angela L. Hollingsworth

Date Issued: 10/07/14 Project Sheet No. 1 Page No. 1 Laboratory Project Identification No. 619-140

STUDY TITLE: Efficacy Evaluation of Copper Enhanced Hard Surfaces as a Sanitizer

STUDY DIRECTOR: Angela L. Hollingsworth

Signature 10/7/14 Date

TEST AND CONTROL ARTICLES:	**LOT NO:**	**DATE RECEIVED:**	**DS NO:**
18 Month Field Use Sample	BEIBD0001-001	08/29/14	E396
Negative Control	Not applicable	08/29/14	E395

PERFORMING DEPARTMENT: Applied Microbiology Laboratory

STORAGE CONDITIONS: Location: K2
■ Dark ■ Ambient Room Temperature
☐ Desiccator ☐ Freezer ☐ Refrigerator ☐ Other:

PROTECTIVE PRECAUTION REQUIRED: MSDS ☐ Yes / ■ No

PHYSICAL DESCRIPTION: ■ Solid ☐ Liquid ☐ Aerosol ☐ Other:

PURPOSE: See attached protocol. **AUTHORIZATION:** See client signature.

PROPOSED EXPERIMENTAL START DATE: 10/07/14 **TERMINATION DATE:** 10/09/14

CONDUCT OF STUDY: ☐ FDA ☐ EPA ☐ R&D ■ GLP ☐ GCP ■ Other: CAL DPR (per EPA GLP)

SPONSOR:	Cupron Inc. 800 East Leigh Street, Suite 123 Richmond, VA 23219	**CONTACT PERSON:** Phone: E-mail:	Alastair B. Monk, PhD 804-381-5514 amonk@cupron.com

TEST CONDITIONS:

Challenge organism(s):	*Staphylococcus aureus*, ATCC 6538
Active ingredient(s):	Copper oxide
Neutralizer(s):	Letheen Broth – 2X
Contact Time(s):	2 hours
Contact Temperature(s):	Ambient (20±1°C)
Dilution(s):	Ready to Use
Organic Load:	■ Yes / ☐ No (Per the protocol to achieve 5% in the inoculum)
Incubation Time(s):	48±4 hours
Incubation Temperature(s):	35-37°C
Comments:	The Test article Lot No. in the Miscellaneous Information Section of the protocol is identified as Lot No. 0001-001. Per the labeling of the materials as received, the Lot No. is as identified on this project sheet above (Lot No. BEIBD0001-001).

Date Issued: 10/15/14	Project Sheet No. 2	Page No. 1	Laboratory Project Identification No. 619-140

STUDY TITLE: Efficacy Evaluation of Copper Enhanced Hard Surfaces as a Sanitizer

STUDY DIRECTOR: Angela L. Hollingsworth

Signature 10/15/14 Date

TEST AND CONTROL ARTICLES:	**LOT NO:**	**DATE RECEIVED:**	**DS NO:**
18 Month Field Use Sample University of Virginia	BEIBD0001-001	08/29/14	E396
Negative Control	Not applicable	08/29/14	E395

PERFORMING DEPARTMENT:
Applied Microbiology Laboratory

STORAGE CONDITIONS: Location: K2
■ Dark ■ Ambient Room Temperature
☐ Desiccator ☐ Freezer ☐ Refrigerator ☐ Other:

CONDUCT OF STUDY: ☐ FDA ☐ EPA ☐ R&D ■ GLP ☐ GCP ■ Other: CAL DPR (per EPA GLP)

SPONSOR: Cupron Inc.
800 East Leigh Street, Suite 123
Richmond, VA 23219

CONTACT PERSON: Alastair B. Monk, PhD
Phone: 804-381-5514
E-mail: amonk@cupron.com

EXPLANATION:

Protocol Amendment(s):

1. The Miscellaneous Information section of the protocol identifies the lot number of the test material as 00001-01. As indicated on Project Sheet No. 1 and above, the correct identifier is BEIBD0001-001.

2. The Miscellaneous Information section of the protocol identifies the test surface as 18 Month Field Use Sample. Per the sponsor, for reporting purposes, the sample will be identified as 18 Month Field Use Sample University of Virginia as outlined above.